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TECHNICAL RESEARCH PROGRESS REPORT

Principal Investigator: Jian Cao, M.D.
Mentor: Stanley Zucker, M.D.
Grant Number: DAMD17-98-1-8160
Project Title: Examination of the role of membrane type-1 matrix metalloproteinase (MT1-MMP) in breast cancer metastasis

Recent studies have demonstrated that the production of tissue degrading enzymes (proteinases) by cancer cells lead to cancer dissemination. Matrix metalloproteinases (MMPs) are the major tissue degrading enzymes responsible for metastasis. Among these enzymes, membrane type matrix metalloproteinases are recently described MMP family members. In contrast to other MMPs, MT-MMPs are anchored in the plasma membrane and activate progelatinase A (also named MMP-2). Since gelatinase A is the MMP most commonly incriminated in breast cancer metastasis, the novel function of MT-MMPs appears important in breast cancer. Because of its central role in cell surface proteolysis, a more complete understanding of the role of MT-MMPs in cancer will expedite progress in therapy of metastasis. To this end, this project has been focused on the role of MT1-MMP in experimental breast cancer invasion and metastasis.

Task 1. Examination of the role of wild type membrane type-1 matrix metalloproteinase (MT1-MMP) in breast cancer metastasis

The study for the examination of the role of wild-type MT1-MMP in breast cancer metastasis has been done during the first two years as scheduled in this fellowship grant. The accomplishment for this section was reported to USAMR last year. In summary, a stable cell line expressing MT/GFP fusion protein was established in a human breast cancer cell line, MDA-MB-436. The cells stably expressing MT/GFP resulted in progelatinase A activation. The localization of MT/GFP in cells was found primarily in the endoplasmic reticulum and in the perinuclear Golgi apparatus. Fluorescence on the leading edge of plasma membrane was also observed but seems limited. By immunofluorescent study using MT1-MMP antibody and TRITC conjugated secondary antibody, the cell surface of MT1-MMP were confirmed. These data indicated that MT1-MMP primarily accumulated in the perinuclear region of transfected cells and may require a signaling mechanism to

facilitate membrane trafficking; GFP does not change the distribution of fused protein. Using GFP as a tag molecule to trace the invasive ability of MDA-MB-436 stable expressing MT1-MMP, I found MT-MMP enhanced the invasive ability of breast cancer cells more than two-fold compared with vector transfected cells. Native MDA-MB-436 cells produce slow growing, poorly invasive tumors. Injection of MDA-MB-436 cells stably transfected with MT1-MMP/GFP cDNA into the inferior mammary fat pad of female nude mice resulted in enhancement of tumor growth and local metastasis as compared to GFP alone transfected tumor cells. Thus, MT1-MMP may trigger the invasion of breast cancer cells by activating progelatinase A and thereby enhance tumor metastasis.

Task 2. Determine whether soluble forms of MT1-MMP play a role in experiment breast cancer dissemination (months 18-30)

To examine whether soluble form of MT1-MMP play a role in experimental breast cancer dissemination, we made a soluble MT1-MMP lacking the C-terminal transmembrane domain and cytoplasmic tail fused with GFP cDNA as a fusion protein. Without the transmembrane domain of MT1-MMP, this chimera is able to secrete into culture medium and is no longer anchored on the plasma membrane examined by immunofluorescent assay. To directly investigate the substrate specificity of MTΔTM, we purified the soluble form of MT1-MMP by using a His tag. A cDNA encoding the His tag was fused to the C-terminus of mutant MT1-MMP lacking the transmembrane domain as a chimera MTΔTM/His. MTΔTM/His purified from transfected COS-1 cells was appeared as 63kDa and 57 kDa proteins in conditioned medium and only 57 kDa form of MTΔTM/His (active form of MT1-MMP) has enzymatic activity against gelatin examined by gelatin zymography. MTΔTM/His is also able to digest type I and type IV collagen by using commercially available fluorescent labeled substrates. It was also noted that purified MTΔTM/His can activate progelatinase A. Therefore, soluble form MT1-MMP appears important role in the degradation of the component of basement membrane. Currently, stable cell lines for both MTΔTM/GFP and MTΔTM/His are being selected for stable expression in MDA-MB-436 cells and CHO-K1 cells, respectively. The role of soluble form of MT1-MMP in breast cancer metastasis will be determined in Boyden chambers and in nude mice.

Task 3. Investigate the trafficking and localization of MT1-MMP in transfected breast cancer cells (months 25-36)

A) We have demonstrated that Con A induces rapid activation of progelatinase A and rapid appearance of MT1-MMP at the cell surface by gelatin zymogram and cell surface biotinylation techniques, respectively in HT1080 cells. By employing same approach along with immunohistochemistry, I will examine the trafficking of MT-MMP in human breast cancer cells, MDA-MB-436. As initially planned, their task will be performed in year 3.

B) Pro-sequence of MT1-MMP serves as an intramolecular chaperone for the production of active MT1-MMP in transfected COS-1 cells.

The goal of this study is to further clarify the role of the propeptide domain of MT1-MMP trafficking to the plasma membrane and in maintaining the function of the plasma membrane-inserted enzyme. We propose that the propeptide sequence of MT1-MMP serves as an intramolecular chaperone in protein folding. To explore the concept, we have co-transfected COS-1 cells with the plasmids encoding the N-terminal propeptide domain of MT1-MMP and the MT1-MMP cDNA lacking the entire propeptide sequence and have demonstrated reconstitution of function of MT1-MMP. Co-tranfection of COS-1 cells with both expression vectors resulted in reconstitution of MT1-MMP function in terms of facilitating ^{125}I labeled TIMP-2 binding to transfected COS-1 cells and subsequent activation of progelatinase A. Transfection of cells with either cDNA alone or cDNA for the propeptide of collagenase-1 with MTΔpro resulted in non-functional cells. Smaller cDNA mutations of the open reading frame of the N-terminal propeptide of MT1-MMP were employed to delineate critical conserved regions of the molecule required as a membrane-bound enzyme. These data indicated that the MT1-MMP prosequence acts as an intramolecualr chaperone and is necessary for the correct folding of the MT1-MMP in vivo.

Task 4. This postdoctoral fellowship grant provides me with importantexperience of studying experimental animal models of breast cancer and in evaluating the effect of mutating MT1-MMP cDNA on the frequency of experimental metastasis. More importantly, Dr. Zucker meets me for 2 and a half hours per week to discuss experimental plans and to analyze

the results from completed experiments. This experience gave me the opportunity to learn how to become an independent investigator using molecular biology techniques to study the role of MMPs in tumor invasion and metastasis.

Relevant publication directly supported by this fellowship grant:

Cao, J., Hymowitz, M., Conner, C., Bahou, W., Zucker, S. The propeptide domain of membrane type-1 matrix metalloproteinase acts as an intramolecular chaperone when expressed in trans with the mature sequence in COS-1 cells. J. Biol. Chem. (in press) October, 2000